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Phenolic Acids Are in Vivo Atheroprotective Compounds Appearing in the Serum of Rats after Blueberry Consumption

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ABSTRACT: Blueberries (BB) have recently been shown to have cardioprotective effects and to prevent atherosclerosis in rodent models. However, the bioactive compounds in BB responsible for these effects have not yet been characterized. Seven phenolic acids (7PA) were identified as metabolites in the serum of rats fed diets supplemented with 10% freeze-dried BB. In this study, 7PA were evaluated for their potential atheroprotective effects in murine macrophage cell line RAW 264.7. 7PA were found to inhibit LPS-induced mRNA expression and protein levels of pro-inflammatory cytokine TNF-α and IL-6 by reducing MAPK JNK, p38, and Erk1/2 phosphorylation. After treatment with 7PA for 2 weeks, mRNA expression and protein levels of scavenger receptor CD36 were decreased ($P < 0.05$), whereas type A scavenger receptor (SR-A) remained unchanged. Moreover, foam cell formation induced by oxLDL and oxLDL binding to macrophages was also inhibited by 7PA. In addition, 7PA increased ($P < 0.05$) expression and protein levels of ATP-binding cassette transporter A1 (ABCA1), which facilitates cholesterol efflux and reduces cholesterol accumulation in macrophages. In summary, the present study demonstrates that certain phenolic acids are potential in vivo atheroprotective compounds following BB consumption in the rodent model. Because BB contain many phytochemicals, other as yet unidentified bioactive compounds may also be important in preventing atherosclerosis in this model and, possibly, in humans.

KEYWORDS: blueberry, cytokine, inflammation, MAPK, phenolic acid, scavenger receptor

INTRODUCTION

It has long been recognized that a diet rich in fruits and vegetables has protective effects on various chronic diseases including cardiovascular diseases (CVD).[1−4] Fruits and vegetables are rich in nutrients and non-nutrient phytochemicals. In recent years, several lines of evidence have accumulated suggesting that polyphenolic compounds may be largely responsible for the cardioprotective effects of colored fruits, mainly through antioxidant and anti-inflammatory actions.[5−8]

Blueberry (BB) is among the most commonly consumed berries in the United States. Currently, BB juice is ranked fourth in consumption after orange juice, grape juice, and pomegranate juice.[9] BB contains a high level of polyphenols and exhibits strong antioxidant and potential anti-inflammatory effects.[10,11] In two recently published papers, BB-enriched diets have shown cardioprotective effects in rats.[12,13] We observed that apoE-deficient mice (apoE$^{-/-}$) fed 1% freeze-dried BB in the diet developed significantly fewer atherosclerotic lesions in the aorta.[14] In follow-up studies, we further showed that BB inhibited pro-inflammatory cytokine production,[15] inhibited scavenger receptor CD36 and SR-A expression, and attenuated foam cell formation.[16] We have also shown that serum polyphenol-enriched extracts made from the sera of rats following BB consumption contained promising atheroprotective components.[15] However, the actual in vivo bioactive compounds responsible for the protective effects of BB have not been characterized.

Polyphenols are widely accepted as the major bioactive compounds in BB. Nonetheless, their roles in vivo are questionable because of their low bioavailability.[7,17] In addition, it is well-known that polyphenols undergo extensive degradation by microflora in the gastrointestinal (GI) tract to form simple phenolic compounds before they are absorbed.[18] Thus, the majority of compounds in circulation and that are exposed to target tissues following polyphenol consumption are likely to be metabolites instead of intact compounds in BB. In our previous study, seven phenolic acids (7PA) were identified as in vivo metabolites following lowbush BB consumption in rats[15] (Figure 1). These 7PA were shown to be at least partly responsible for the observed health effects on bone growth promotion observed in lowbush BB fed rats.[19] In the present study, we investigated the possible atheroprotective effects of 7PA in a murine macrophage cell line RAW 264.7. On the basis of the results from our previous studies,[15,16] we focused on the possible effects of 7PA in reducing pro-inflammatory cytokine production and inhibiting scavenger receptor expression.

MATERIALS AND METHODS

Chemicals and Reagents. Hippuric acid, 3-hydroxyphenylacetic acid, 3-hydroxybenzoic acid, ferulic acid, 3-(4-hydroxyphenyl)propionic acid, and 3-hydroxycinnamic acid were obtained from Sigma (St. Louis, MO). 3-(3-Hydroxyphenyl)propionic acid was purchased from Alfa Aesar (Ward Hill, MA). Anti-CD36 mAb, anti-ABCA1 mAb, and anti-β-actin mAb were purchased from Cayman (Ann Arbor, MI), Abcam (Cambridge, MA), and Sigma (St. Louis, MO), respectively. LPS, zeocin, and QuantiBlue (an alkaline phosphatase substrate) were purchased from

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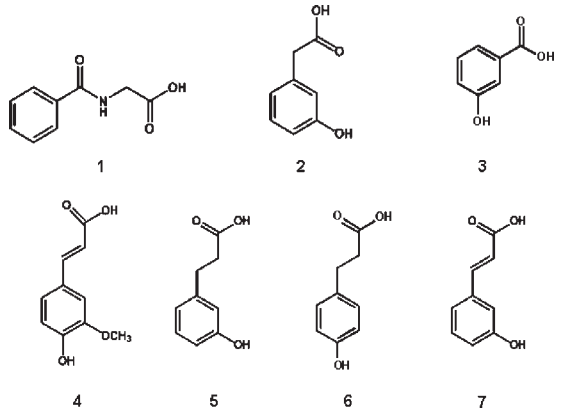

Figure 1. Chemical structures of seven phenolic acids identified as in vivo metabolites following blueberry consumption in rats (compounds **1**−**7** are hippuric acid, 3-hydroxyphenylacetic acid, 3-hydroxybenzoic acid, ferulic acid, 3-(3-hydroxyphenyl)propionic acid, 3-(4-hydroxyphenyl)propionic acid, and 3-hydroxycinnamic acid, respectively).

Table 1. Concentrations (Nanograms per Deciliter) of Seven Phenolic Acids Used in This Study To Treat Cells

compound	control	1× 7PA	2× 7PA	8× 7PA
hippuric acid (**1**)	8.68	59.80	119.60	478.40
3-hydroxyphenylacetic acid (**2**)	0.32	2.59	5.18	20.72
3-hydroxybenzoic acid (**3**)	0	0.34	0.68	2.72
ferulic acid (**4**)	0.01	0.09	0.18	0.72
3-(3-hydroxyphenyl)propionic acid (**5**)	1.05	10.64	21.28	85.12
3-(4-hydroxyphenyl)propionic acid (**6**)	0.02	0.15	0.30	1.20
3-hydroxycinnamic acid (**7**)	0.05	0.47	0.94	3.76

Invivogen (San Diego, CA). All solvents were obtained from Fisher Scientific (Pittsburgh, PA).

Stock solutions of 7PA mixture in DMSO were made 10000-fold higher than the concentrations previously reported in the sera of rats fed control casein diet or control diet supplemented with 10% BB.[15]

Cell Culture. RAW 264.7 macrophages and (Invivogen, San Diego, CA) were cultured in DMEM supplemented with 10% (v/v) FBS (Hyclone, Logan, UT) and zeocineosin (200 μg/mL). Cells were treated with a mixture of 7PA at three doses (Table 1): the concentrations of the 7PA mixture were equal to that in serum of rats fed 10% BB (1× 7PA), 2-fold higher (2× 7PA), or 8-fold higher (8× 7PA). The seven-compound mixture with the same concentration in the serum of rats fed control diet was served as control (Con). For long-term treatment, RAW 264.7 cells were treated with 1× 7PA daily for 2 weeks (four sequential passages); cell culture medium was changed every 2 days.

RAW-Blue cells (Invitrogen, San Diego, CA) are derived from RAW 264.7 macrophages with chromosomal integration of a SEAP reporter construct inducible by NF-κB and AP-1. RAW-Blue mouse macrophage cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) (Hyclone) and zeocineosin (200 μg/mL). RAW-Blue cells were used in LPS-induced secreted embryonic alkaline phosphatase (SEAP) reporter assay. The SEAP reporter assay was designed to measure nuclear factor-κB (NF-κB) activation.[20,21]

Real-Time RT-PCR Analysis. Total RNA was isolated using Trizol reagent (Invitrogen) according to the manufacturer's instructions and treated with RNase-free DNase. Procedures of reverse transcription reaction and quantitative real-time PCR were described previously.[22] Real-time PCR primers (from Integrated DNA Technologies, Coralville, IA) were as follows: β-actin sense, GGCTATGCTCTCCCTCACG; β-actin antisense, CGCTCGGTCAGGATCTTCAT; TNF-α sense, ACAAGGCTGCCCCGACTAC; TNF-α antisense, TGGAAGACTCC-TCCCAGGTATATG; IL-6 sense, TGGAGTCACAGAAGGAGTGGC-TAAG; IL-6 antisense, TCTGACCACAGTGAGGAATGTCCAC; SR-A sense, ATGAACGAGAGGATGCTGACTG; SR-A antisense, GTGCT-GTGAGGAAGGGATGC. Primers of CD36 were obtained from SA-Bioscience (catalog no. PPM03796B, Frederick, MD).

TNF-α and IL-6 ELISA. RAW 264.7 cells (5×10^6cells/well) were pretreated with various concentrations of the indicated reagents for 1 h before LPS stimulation. After 18 h of LPS stimulation, supernatant was collected; TNF-α and IL-6 in the supernatant was determined by ELISA using Duoset ELISA kits (R&D, Minneapolis, MN) according to the manufacturer's instructions. The optical density was determined using a BMG PolarStar microplate reader (BMG Labtech, Durham, NC) at 450 nm. A mean value of triplicate samples for each experiment and two separate experiments were used for analysis.

Western Blot Analysis. RAW 264.7 cells treated with indicated reagents were lysed in RIPA buffer (Cell Signaling, Danvers, MA). Cell lysates were centrifuged at 5500*g* for 15 min to remove cell debris. Protein concentrations were determined by Bio-Rad protein assay reagent (Bio-Rad, Richmond, CA). The lysate (10 μg of protein/lane) was separated by SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were blocked with 5% BSA/PBS containing 0.05% Tween-20 and probed with specific antibodies and β-actin. Bands were detected using ECL reagents (GE Healthcare, Piscataway, NJ) according to the manufacturer's instructions.

SEAP Reporter Assay. RAW-Blue cells (1×10^5 cells/well) were treated with control and three doses of 7PA for 18 h. The supernatants were then collected for SEAP secretion assay. QUANTI-Blue powder was dissolved in endotoxin-free water and sterile filtered (0.22 μm) (QuantiQuanta-Blue substrate). RAW-Blue cell supernatant (40 μL/well) was added to QuantiQuanta-Blue substrate (160 μL/well) and incubated at 37 °C for 0.5−1 h. Absorbance was measured at 620 nm in a PolarStar microplate reader (BMG Labtech, Durham, NC).

Signaling Analysis. Cells were lysed in RIPA buffer with protease and phosphatase inhibitors (Cell Signaling). Cell lysates were centrifuged at 5500*g* for 15 min to remove cell debris. Protein concentrations were determined by Bio-Rad protein assay reagent. The lysates were used for signaling analysis by two PathScan Sandwich ELISA Kits (Cell Signaling): PathScan Multi-Target Sandwich ELISA Kit, which was designed to measure phospho-SAPK/JNK and phospho-p38 MAPK, as well as PathScan Total p44/42 MAPK (Erk1/2) Sandwich ELISA Kit for phospho-p44/42. The assays were conducted according to the manufacturer's protocol.

OxLDL-Induced Foam Cell Formation. For foam cell assay, cells were pretreated with 1× 7PA for 2 weeks followed by incubation with 10 μg/mL oxLDL for 24 h. Cells were then fixed and stained with Oil Red O and 4′,6-diamidino-2-phenylindole (DAPI) mount. To determine percent foam cells, the total numbers of cells (DIPA+) and foam cells (Oil Red O+) were counted in five separate fields using an Olympus fluorescence microscope (Olympus, Tokyo, Japan).

Flow Cytometry Analysis. RAW 264.7 cells (5×10^5) were incubated with corresponding mAbs or mIgA or mIgG at 4 °C for 1 h, followed by incubation with oxLDL (10 μg/mL) at 4 °C for 1 h. OxLDL binding was detected using rabbit anti-apoB IgG biotin (3 μg/mL) and saturating concentrations of streptavidin-PE (Jackson ImmunoResearch, West Grove, PA). CD36 and ABCA1 proteins were detected using R-Phycoerythrin AffiniPure F(ab′)2 fragment donkey anti-mouse IgG (Jackson ImmunoResearch). Cells were washed with PBS/1% BSA, fixed in PBS/1% formalin, and acquired in a FACSCalibur flow cytometer and analyzed using CellQuestPro software (BD-Biosciences, San Jose, CA).

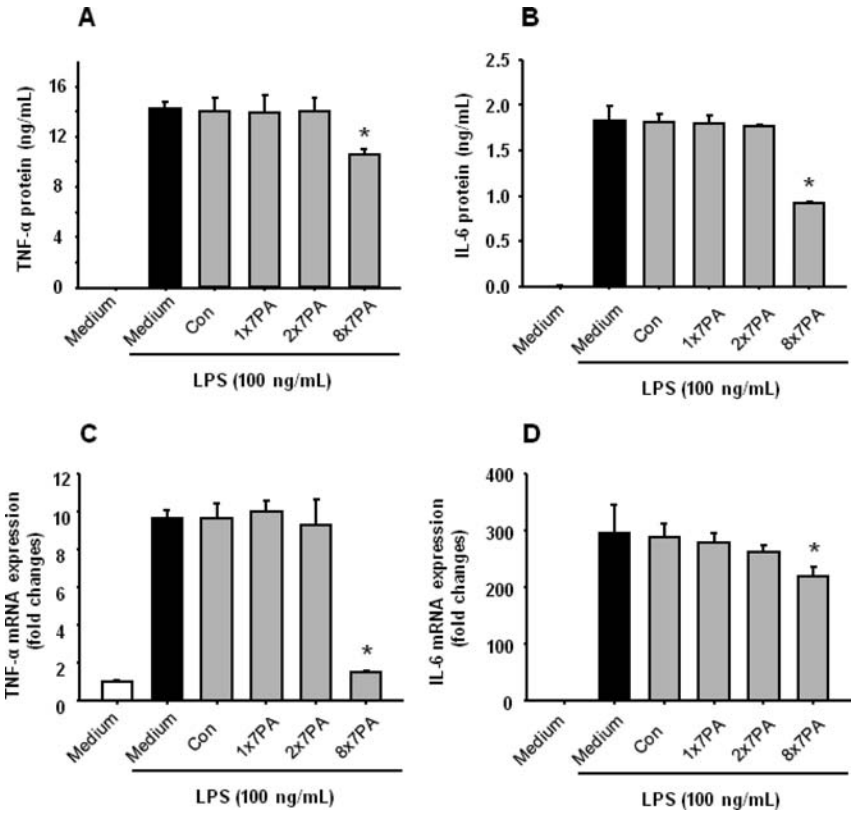

Figure 2. Inhibitory effects of three doses of 7PA in mRNA expression and protein levels of pro-inflammatory cytokine TNF-α and IL-6 in LPS-induced macrophages. 8× 7PA, after being treated cells for 1 h, significantly reduced protein levels (A, B) and down-regulated mRNA expression (C, D) of TNF-α and IL-6 in RAW 264.7 macrophages (mean ± SEM; ∗, $P < 0.05$).

Statistical Analysis. Data were expressed as the mean value ± SEM. Three or four replicates were used in the study. Student's *t* test was used to analyze differences between groups. One-way ANOVA with the Student−Newman−Keuls method was used to compare more than two groups. A value of $P < 0.05$ was considered to be significant difference unless otherwise mentioned. Statistical analyses were performed by SigmaStat statistical software (SigmaStat 3.5, San Jose, CA).

RESULTS

7PA Down-regulate LPS-Induced Gene Expression and Protein Levels of IL-6 and TNF-α in Macrophages. RAW 264.7 macrophages were treated with control and three doses of 7PA (1×, 2×, and 8×) followed by LPS stimulation. Gene expression and protein levels of IL-6 and TNF-α were lower only at 8× 7PA treatment ($P < 0.05$) (Figure 2). Macrophages treated with control and lower concentrations of 7PA (1× and 2×) did not alter gene expression and protein levels of IL-6 and TNF-α.

7PA Did Not Affect NF-κB Activation Assessed by SEAP Reporter Assay. RAW 264.7 macrophages were treated with three doses of 7PA (1×, 2×, and 8×) and control. The cells were used in SEAP reporter assay for measuring NF-κB activation. Compared with the control, cells treated with three different doses of 7PA did not show any differences (data not shown). This indicated that 7PA had no effects in inhibiting activation of the NF-κB pathway.

7PA Inhibited LPS-Induced Phosphorylation of p38, JNK, and Erk1/2. To investigate the signaling pathways involved in the production of TNF-α and IL-6, two commercial PathScan ELISA kits were employed to check the effects of 7PA in affecting MAP kinase pathways. RAW 264.7 cells were pretreated with 8× 7PA for 1 h and stimulated with LPS for 30 min. 8× 7PA selectively inhibited p38, JNK, and Erk1/2 phosphorylation, whereas control had no effects on the phosphorylation of either (Figure 3).

7PA Inhibited Expression of Scavenger Receptor CD36. RAW 264.7 macrophages were treated with control and three doses of 7PA (1×, 2×, and 8×). CD36 and SR-A mRNA expression and protein levels were determined using RT-PCR and Western blot. There were no differences in mRNA expression and protein levels of CD36 and SR-A between control and three doses of 7PA treatments following acute treatment (2, 6, and 18 h after single treatment) (data not shown). However, with longer term treatment using 1× 7PA for 2 weeks, CD36 mRNA expression and protein levels were lower in the 1× 7PA group ($P < 0.05$), whereas those of SR-A remained unchanged (Figure 4).

7PA Attenuated Foam Cell Formation. OxLDL-facilitated foam cell formation in RAW 264.7 macrophages was attenuated ($P < 0.05$) by 1× 7PA treatment for 2 weeks compared with control treatment (Figure 5). Foam cells are indicated by the intense red color after Oil Red O staining.

7PA Reduced OxLDL Binding to Macrophages and CD36 Protein Levels. Flow cytometry was used to assess oxLDL binding to macrophages and the CD36 protein levels in RAW 264.7 macrophages. 1× 7PA treatment for 2 weeks resulted in less oxLDL binding to macrophages as compared with control ($P < 0.05$) (Figure 6A,B). Flow cytometry analysis confirmed a decrease ($P < 0.05$) of CD36 protein levels in cells treated with 1× 7PA compared with control (Figure 6C,D).

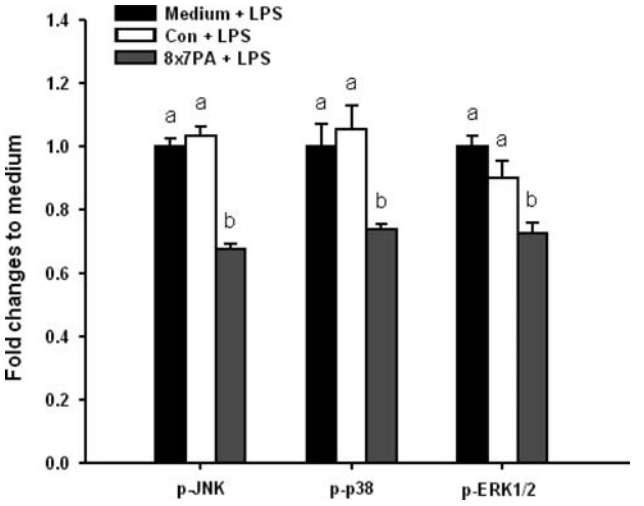

Figure 3. 8× 7PA inhibited p38, JNK, and Erk1/2 phosphorylation in LPS-induced RAW 264.7 macrophages. Means with different letters are significantly different (mean ± SEM, $P < 0.05$).

7PA Up-regulated ABCA1 Expression and Increased Protein Level. 1× 7PA treatment for 2 weeks increased expression of ATP-binding cassette transporter A1 (ABCA1) in RAW 264.7 macrophages ($P < 0.05$) (Figure 7A). The protein level of ABCA1, which was analyzed by flow cytometry, was also elevated (Figure 7B,C).

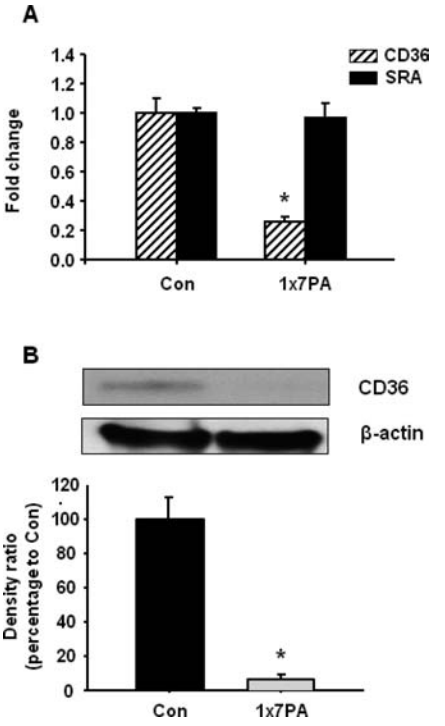

Figure 4. After treatment with control and 1× 7PA for 2 weeks, CD36 mRNA expression (A) and protein level (B) in RAW 264.7 macrophages were significantly low in the 1× 7PA group, whereas SR-A remained unchanged (mean ± SEM; ∗, $P < 0.05$).

DISCUSSION

Identifying bioactive compounds in foods and establishing their health effects are active areas in nutritional and food research.[23] However, the primary challenge in this area is the number and diversity of compounds in a given food. Absorption, metabolism, and biotransformation by gut microflora are key factors to link the in vivo biological activity and the bioactive compounds in foods.

BB contains high levels of polyphenols including anthocyanins, proanthocyanidins, PAs, and other phenolic compounds.[24−29] Polyphenols are widely considered as major bioactive compounds in BB responsible for protective effects against vascular diseases.[5,30,31] The absorption of polyphenols is an essential step in determining their biological activity and effects on health. Studies have clearly shown that the major polyphenols in BB including anthocyanins and proanthocyandins were poorly absorbed.[7,32] Only a very small proportion of polyphenols in BB is absorbed intact.[33] On the other hand, it is well-known that polyphenols undergo extensive degradation by gut microflora to form various lower molecular weight catabolites that could be absorbed after biotransformation by the colon microflora. The main catabolites in the colon are various PAs and their derivatives, such as benzoic acids (C6−C1), phenylacetic acids (C6−C2), and phenylpropionic acids (C6−C3).[18] Many of these catabolites are efficiently absorbed in the colon, appear in the blood, and enter target tissues. As a result, some of these compounds can reach a considerable concentration in vivo. Because microbial catabolites may be present at many sites of the body in higher concentration than the parent compounds, it is proposed that at least a part of the biological activities ascribed to berry polyphenols are due to their colonic catabolites.[18] In our previous study,[19] 7PA (Figure 1), which appeared to be colonic catabolites of polyphenols, were found as in vivo metabolites in serum of rats fed 10% lowbush BB in the diet. 7PA were shown to be at least partly responsible for the health effects in promoting bone growth observed in BB-fed rats.[19] The possible atheroprotective effects of 7PA, especially their ability in inhibiting pro-inflammatory cytokine production and scavenger receptor CD36 and SR-A expression,[15,16] were evaluated in this study.

To investigate their effects in inhibiting pro-inflammatory cytokine expression and production, a dose−response experiment was performed. The doses were determined by assessing 7PA concentrations in serum of rats fed no BB (control) and in rats fed 10% BB-containing diets.[15] The 7PA concentrations studied were 1-, 2-, and 8-fold the mean concentration found in the BB-fed rats. Only the dose of 8× 7PA had significant effects in inhibiting TNF-α and IL-6 mRNA expression and protein levels compared with control (Figure 2). It is worth mentioning that the peak concentrations of these PAs in BB-fed rats were probably much higher than the concentrations measured at sacrifice (∼6−9 h following the last food consumption). Thus, the 8-fold level used in these studies is probably well within the peak concentration range achieved following BB consumption. Another important issue is that the effects observed in animals occur after long-term feeding of 20 weeks.[14] In the current experiment, only acute treatment was used, so it is not surprising that a higher dose was required to exhibit these effects in vitro.

Several potential mechanisms are involved in the inhibition of LPS-induced inflammatory cytokine production, of which blockades of NF-κB and MAPK pathways have been proposed as the two major mechanisms. Activation of MAPK and NF-κB leads to the production of inflammatory cytokines.[34−36] We have previously shown that serum polyphenol-rich extracts from rats fed

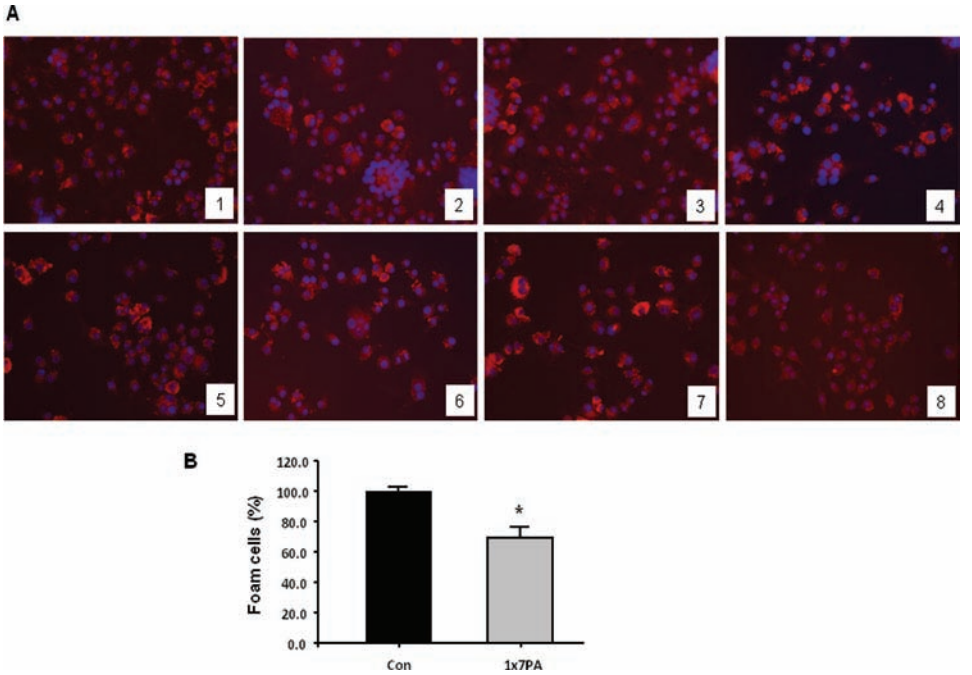

Figure 5. After treatment with control and 1× 7PA for 2 weeks, RAW 264.7 macrophages were incubated with oxLDL to check foam cell formation. Compared to cells treated with control (A1−4), the 1× 7PA treatment attenuated foam cell formation (A4−8, B) (mean ± SEM; ∗, $P < 0.05$). Foam cells are indicated by the intense red color after Oil Red O staining.

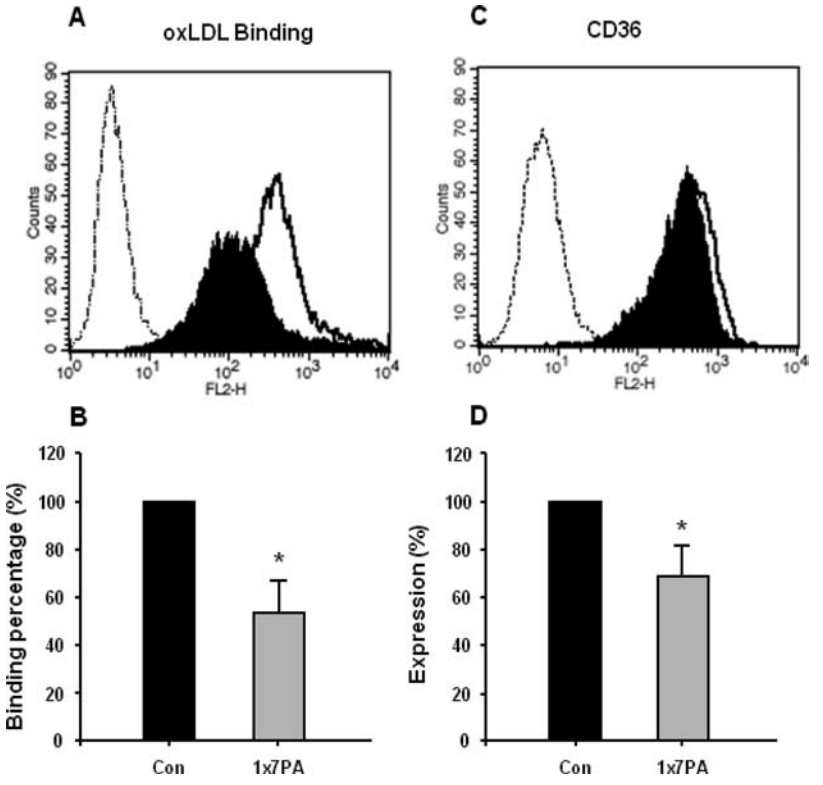

Figure 6. OxLDL binding to macrophages and CD36 protein levels assessed by flow cytometry after RAW 264.7 macrophages were treated with control and 1× 7PA for 2 weeks. 1× 7PA treatment resulted in the reduction of oxLDL binding to macrophages by about 50% compared to control (A, B). The binding percentage was calculated on the basis of the fluorescence intensities. The CD36 protein level was also lower in cells treated with 1× 7PA (C, D) (mean ± SEM; ∗, $P < 0.05$).

10% BB reduced phosphorylation of IκB, NF-κB p65, MAPK p38, and JNK in vitro.[15] Data presented in this study suggest that 7PA did not affect NF-κB activation assessed by SEAP reporter assay. However, 8× 7PA did significantly inhibit phosphorylation

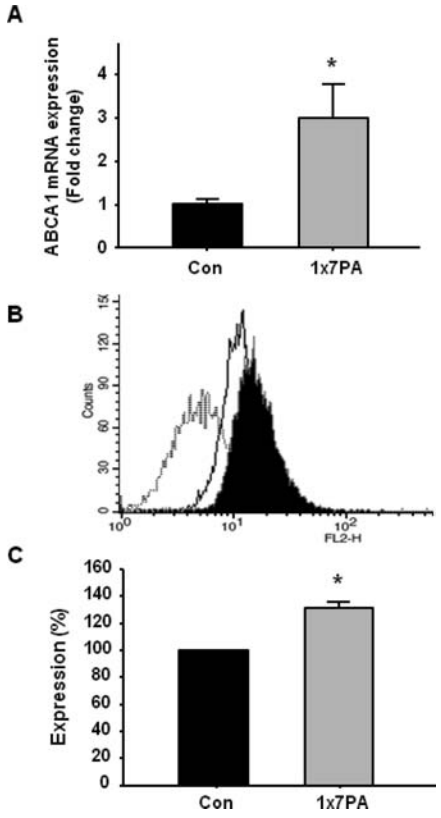

Figure 7. After RAW 264.7 macrophages were treated with control and 1× 7PA for 2 weeks. 1× 7PA significantly increased the mRNA expression of ABCA1 (A). The protein level of ABCA1 was also elevated as analyzed by flow cytometry (B, C) (∗, $P < 0.05$).

of MAPK p38, JNK, and Erk1/2, indicating that all three major components in the MAPK pathway were affected by 7PA.

Besides reducing pro-inflammatory cytokine production, BB feeding has also been shown to inhibit two important scavenger receptors, CD36 and SR-A, and to attenuate foam cell formation.[16] However, acute treatment with 1× 7PA to 8× 7PA up to 18 h did not change CD36 and SR-A expression. To imitate the long-term feeding pattern in mice,[16] RAW 264.7 macrophages were treated with 1× PA for 2 weeks. CD36 was found to be significantly down-regulated, whereas SR-A remained unchanged. In addition, fewer foam cells developed when induced with oxLDL following 2 weeks of treatment with 1× 7PA (Figure 5). Further measurement by flow cytometry showed that long-term treatment of 2 weeks with 1× 7PA reduced binding of oxLDL to macrophages and confirmed the lower protein level of CD36 in macrophages. Hence, 7PA were able to down-regulate CD36 expression and reduce foam cell formation. These effects were achieved only after long-term exposure to 7PA at low dose. The potential mechanisms, especially the effects of 7PA on the PPARγ pathway, are currently under investigation in our laboratory.

ABCA1 is a cholesterol efflux regulatory protein (CERP) encoded in rodents and humans by the ABCA1 gene and a major regulator of cellular phospholipids and cholesterol homeostasis. Lack of this gene is associated with familial HDL deficiency. ABCA1 is a key transporter to facilitate cholesterol efflux and reduce cholesterol accumulation in macrophages.[37] In this study, after macrophages were treated with 1× PA for 2 weeks, both mRNA expression and protein levels of ABCA1 were significantly increased, suggesting that 7PA could up-regulate ABCA1 and contribute to cholesterol efflux. Because ABCA1 expression is known to be regulated via the nuclear receptor LXR, potential interaction of 7PA with LXR signaling requires further investigation.

On the basis of the results from this study, the mixture of 7PA exhibited potential atheroprotective effects in the macrophages; thus, they should be considered as potential in vivo bioactive compounds. Nevertheless, they represented only certain aspects of the protective effects observed from whole BB feeding in vivo. For example, BB feeding inhibited the NF-κB signaling pathway and expression for both CD36 and SR-A,[16] whereas 7PA inhibited only expression of MAP kinases and CD36. These data suggested that other as yet unidentified bioactive compounds may also be important in preventing atherosclerosis. Another question raised in this study is the bioactivities of each phenolic acid. The bioactivities observed from this study were the effects of a seven-compound mixture, which was intended to imitate the in vivo conditions following BB consumption. However, an important question would be whether these seven compounds worked synergistically, or, in the alternative, that the seven compounds each had an additive effect. Further investigation to address this question is currently being carried out in our laboratory.

Another important fact is that six of the seven PAs already exist in the circulation of rats fed a control diet because they are common end-products of different types of dietary components including certain proteins. However, due to low concentrations, they did not show similar health effects. BB consumption was able to significantly increase the concentration of certain PAs as they contain high levels of polyphenols as precursors of these PAs. Therefore, to some extent, it is the dose rather than the type of compounds that matters when dealing with in vivo bioactive compounds following berry consumption.

In summary, the present study demonstrated that certain phenolic acids are potential in vivo atheroprotective compounds following BB consumption in the rodent model. The underlying mechanisms of the protective effects as well as the effects of each individual compounds warrant further investigation. Because BB contains many phytochemicals, other as yet unidentified bioactive compounds may also be important in preventing atherosclerosis.

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ABBREVIATIONS USED

ABCA1, ATP-binding cassette transporter A1; ApoE$^{-/-}$, apolipoprotein E deficient; BB, blueberries; CD, AIN-93G diet; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; SEAP, secreted alkaline phosphatase; TNF-α, tumor necrosis factor-α; RT-PCR, reverse transcription—polymerase

chain reaction; IL-6, interleukin-6; CD36, cluster of differentiation 36; SR-A, scavenger receptor class A.

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